

An approach to the understanding of the nasal early-phase reaction induced by nasal allergen challenge

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Quantitative determinations of the inflammatory mediators in nasal secretions were performed and correlated with the objective nasal symptoms within 1 h after nasal allergen challenge (NAC). Twenty-six patients with seasonal allergic rhinitis were enrolled outside the pollen season. All measurements were performed before (as a baseline control) and at 1, 5, 10, 30, and 60 min after NAC. This study aimed to clarify the pathogenic mechanism of the early-phase reaction (EPR) by monitoring the evolution of early-phase mediators in nasal secretions and the presence of nasal symptoms during this period. The results showed that, after NAC, the maximal mediator concentration was already reached after 1 min for histamine (124 ng/g), 5 min for tryptase (56 μ U/g), and 5–10 min for leukotriene C₄ (40 ng/g). Itching and sneezing started as early as 20–30 s, and they were predominant symptoms within 5 min. Rhinorrhea and nasal obstruction started a few minutes after NAC and lasted until more than 1 h after NAC. There was no significant correlation between any single mediator and nasal symptoms during the sampling period. In conclusion, this study demonstrated that during the EPR the presence of nasal symptoms involves a complex mechanism, reflecting the interaction between the mediators released by inflammatory cells, and the receptors on different target organs. When evaluating symptoms during the EPR, one must consider not only the severity of these symptoms but also the time period within which these symptoms occur. For the symptoms of nasal obstruction and rhinorrhea, the early-phase reaction often lasted more than 1 h.

Key words: early-phase reaction; mediators; nasal allergen challenge; nasal secretions; nasal symptoms.

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In an intranasal challenge with specific allergens in atopic patients, nasal symptoms such as sneezing, nasal obstruction, and rhinorrhea may occur within minutes. This response has been described as the early-phase reaction (EPR), which usually subsides within 1 h (1). Later, many investigators have observed that a significant increase in mediators such as histamine (2, 3), tryptase (4, 5), prostaglandin D₂ (PGD₂) (2, 3), leukotriene C₄ (LTC₄) (6, 7), and kinins (2, 3) was associated with the nasal symptoms several minutes after the challenge. These observations strongly support the hypothesis of an important role for mast-cell activation during the EPR.

However, the EPR has been assessed merely by measuring the nasal symptoms and/or mediator

concentrations at a certain time (a few minutes) after nasal allergen challenge. The detailed mechanism of the entire EPR is not well understood. Therefore, it is important to study not only the symptoms and mediators of the EPR but also their quantitative variations during the EPR. This could improve our understanding of the pathophysiology of the EPR.

The aim of this study was to study quantitatively the changes within 1 h after nasal allergen challenge (NAC) of several important mediators such as histamine, tryptase, and LTC₄ and to correlate these measures with objective nasal symptoms, i.e., the number of sneezes, the weight of secretions (rhinorrhea), and nasal resistance measured by passive anterior rhinomanometry (PAR).

Material and methods

Study subjects

Twenty-six patients (13 men and 13 women) aged 19–38 years (mean age 24.8 years) had nasal atopy to grass pollen confirmed by medical history and skin test. Fourteen of the 26 patients also had atopy to house-dust mites as shown by skin test. However, they were all symptom-free, and they had normal nasal airway resistance (NAR) confirmed by PAR. In all test subjects, the number of eosinophils in nasal secretions was lower than 6% of the total leukocytes, indicating no obvious inflammation of the nasal mucosa at present (8). All test subjects had been disease- and medication-free (except contraceptives) during the previous 3 months. The study was performed 3 months after the end of the pollen season. All of the study subjects gave their informed consent to participation in this study. The study was approved by the ethics committee of the University Hospital, Free University of Brussels (Belgium).

The aspiration system

The authors used the Biewenga aspiration system (9, 10). The samples were collected by repeated aspiration from the middle meatus and from the floor of both nasal cavities, with a metal tube with an inner diameter of 0.5 mm, into a preweighed plastic sampling tube. This was immediately followed by aspiration of a known volume (1.0 ml) of phosphate-buffered saline (PBS), pH 7.4, including 10% Mesna (Mistabron, sodium 2-mercaptoethane sulfonate, UCB Pharmaceuticals, Brussels, Belgium). Mesna acts by disrupting the disulfide bonds of the mucus polypeptide chains to decrease the viscosity of the samples. This is necessary to enable the pipetting of precise volumes of nasal aspiration fluids. The total amount of secretion sampled was measured by weighing on a balance with a precision of 10 mg the collection tube and the tube with the aliquot of PBS before and after sampling. The individual dilution factor was then calculated.

Nasal allergen challenge (NAC)

NAC was effected by nasal aerosol application with a Heyer nebulizer (10, 11) (Heyer, Bad Ems, Germany) of the grass-pollen mixtures (20 000 AU/ml, pH 7.4, HALAB Allergy Service, Brussels, Belgium). Briefly, the nebulizer contained a provocation extract. The nasal mucosa was consecutively provoked six times over 10 s (three times for each nostril alternately), the study subject being

in complete apnea after full inspiration. This precaution prevented the allergen from entering the bronchial tree. The temperature of the aerosol was maintained at 28–30°C.

One sample was collected before the challenge as a baseline control. NAC was performed 15 min later. Nasal secretions were collected at 1, 5, 10, 30, and 60 min after initial challenge. Six samples were obtained from each test subject. The samples were cooled on ice immediately after sampling, and then mixed in a Vortex mixer and centrifuged at 1000 g at 4°C for 15 min. The supernatants were stored at –20 or –80°C within 1 h after sampling until used.

Measurement of the mediators

Histamine was determined by radioimmunoassay (RIA) with a double-antibody separation system, according to the manufacturer's instructions (Pharmacia histamine RIA, Uppsala, Sweden) (8, 12). Tryptase was determined by immunoradiometric assay (IRMA) with a coated tubal technique, according to the manufacturer's instructions (Pharmacia Tryptase RIACT, Uppsala, Sweden) (8, 12). LTC₄ was determined by enzyme immunoassay (EIA) (EIA kit from Stallergènes, Fresnes, France) (8, 12). After the enzymatic tracer and the specific antiserum had been added to the samples, they were incubated for 18 h at 22°C in subdued light. This was specified in the protocol in order to minimize cross-reaction with LTD₄ (46%), LTE₄ (2%), and LTB₄ (<0.1%). Mesna was added in recovery experiments to evaluate its possible influence and was shown not to influence the results of these determinations.

Objective evaluation

The number of sneezes was recorded in absolute number during each sampling period. Nasal airway resistance (NAR) was measured by PAR (Heyer Company, Germany) expressed in Pa/cm³ per s of left and right sides at each sampling time. Total nasal resistance (R_{tot}) was computed with the equation: $R_{\text{tot}} = R_l \times R_r / (R_l + R_r)$ (13, 14). Nasal secretions were collected by using a preweighed handkerchief during each sampling period. The test subjects were asked to collect the secretions with the handkerchief only when secretions were running out of the nostrils, but without blowing the nose.

Statistical evaluation

All statistical tests were carried out two-tailed at the 5% level of significance. The calculations were performed with the SPSS/PC+ program on an IBM-compatible microcomputer.

First, we calculated the median value of each measurement at different sampling times after NAC in order to determine the evolution of these parameters during EPR. To investigate the relationship between the measurements which were obtained in two different ways, we performed the following transformations. For the variables measured at each sampling time (nasal resistance and concentration of the mediators), the area under the curve (AUC) was used, while for the measurements collected during successive sampling periods (number of sneezes and the weight of secretions), the sum was used. A maximum value and the time of maximum value reached were also calculated for the nasal resistance and the concentration of the mediators. Then, the Spearman correlation coefficients (r_s) were used to quantify the relationship between the variables of nasal symptoms and the concentrations of mediators over the entire sampling period.

Results

Nasal symptoms

Itching and sneezing. Itchy nose could occur at the earliest 20–30 s after NAC and was immediately followed by sneezing. The median number of sneezes was five times (range: 0–22 times) during the first minute and five times (range: 0–17 times) at 1–5 min after NAC. In most patients, itching and sneezing gradually ended about 5 min after challenge (Table 1).

Rhinorrhea. In all patients, rhinorrhea started a few minutes after NAC. Even at 1 h after NAC, rhinorrhea persisted in 24 patients (92%). When the median weight of secretions per minute was considered, a maximum was reached in the period 1–5 min after NAC (Table 1).

Nasal obstruction. In all subjects, bilateral or unilateral nasal obstruction (100% increase in the baseline NAR) was observed at 1–5 min after NAC. The median maximum total nasal resistance was 1.4 Pa/cm³ per s (4.8 times higher than the baseline value) occurring at 10–30 min after NAC (Table 2). One hour after NAC, a median total nasal resistance of 0.68 Pa/cm³ per s could be observed, and that was still much higher (2.3 times) than the median baseline value of 0.29 Pa/cm³ per s (Fig. 1).

Concentrations of mediators (Fig. 1)

Histamine. The median baseline concentration of histamine in the nasal secretions was 29 ng/g

Table 1. Median number of sneezes and weight of secretions recorded before (time zero) and during successive sampling periods after nasal allergen challenge in 26 atopic patients outside pollen season. Median weight of secretions/min was also estimated as follows: total median weight of secretions divided by time (min) of each sampling period

Period (min)	Median no. of sneezes (range)	Median wt. (g) of secretions (range)	Median wt. (g) of secretions/min
0–1	5 (0–22)	0.045 (0–2.1)	0.045
1–5	5 (0–17)	0.280 (0–1.6)	0.070
5–10	0 (0–7)	0.230 (0–2.2)	0.046
10–30	0 (0)	0.705 (0.1–3.7)	0.035
30–60	0 (0)	0.255 (0–2.4)	0.008

(range: 10–94 ng/g). Histamine rapidly increased and reached maximum concentrations (median: 124 ng/g; range: 36–592 ng/g) 1 min after NAC (Table 2). The concentration of histamine started to decrease at 5 min and returned to baseline about 30 min after NAC.

Tryptase. The median baseline concentration of tryptase was under the measurable level (<0.5 µU/g) in most of the patients (median: <0.5 µU/g, range: <0.5–9.1 µU/g). Tryptase concentrations started to increase at 1 min after NAC and reached their maximum concentration (median: 56 µU/g, range: <0.5–500 µU/g) at 5 min after NAC (Table 2). The concentration of tryptase returned to baseline about 30 min after NAC.

Leukotriene C₄. The median baseline concentration of LTC₄ was 5.3 ng/g (range: 0.7–12.7 ng/g). LTC₄ started to increase 1 min after NAC and reached its maximum concentration (median: 40 ng/g, range: 5.2–268 ng/g) at 5–10 min after NAC (Table 2). The concentrations of LTC₄ returned to baseline about 30 min after NAC.

Correlation between the measurements

A significant positive relationship was found only between the number of sneezes and the weight of the secretions ($r_s=0.471$, $P=0.015$), and between the AUC of nasal resistance and the weight of the secretions ($r_s=0.452$, $P=0.02$). No significant correlation was found between the mediators and any of the nasal symptoms.

Discussion

Recently, there has been considerable interest in the late-phase reaction because it explains much of the reactions and complaints of the patients such as hyperreactivity and priming. It might also provide a basis for a more rational use of anti-

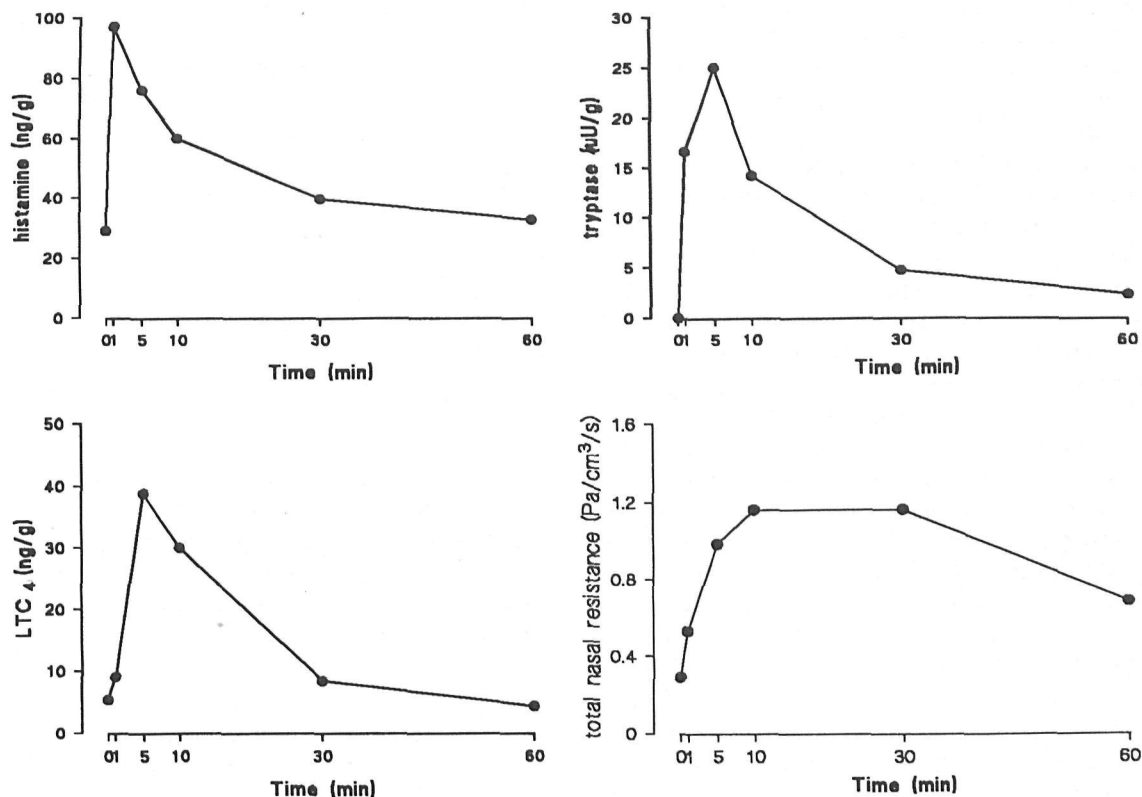


Fig. 1. Median total nasal resistance and concentrations of histamine, tryptase, and LTC₄ in nasal secretions before (time zero) and at different times (min) after nasal allergen challenge in 26 atopic patients outside pollen season. For mediators, baseline recording (time zero) was performed 15 min before challenge.

Table 2. Median maximum values of total nasal resistance, and concentrations of histamine, tryptase, and LTC₄ in nasal secretions and time when these maximum values were reached within 1 h after nasal allergen challenge in 26 atopic patients outside pollen season. Values of number of sneezes and weight of secretions are not applicable, because they were collected only over sampling period (i.e., 0–1 min and 1–5 min)

Nasal symptoms / mediators	Maximum value	Time when maximum reached (min)
Total nasal resistance (Pa/cm ³ per s)	1.4	10–30
Histamine (ng/g)	124	1
Tryptase (μU/g)	56	5
LTC ₄ (ng/g)	40	5–10

inflammatory agents. In fact, the term "late-phase reaction" covers the phenomenon of chronic inflammation, which is predominantly due to eosinophilia in the nasal mucosa of an allergic patient after NAC. However, the nasal late-phase response is considerably weaker than the early-phase response, and a biphasic pattern is not a typical feature (15). On the other hand, natural exposure to pollen in the season may consist of an average of 15 000 tiny challenges per day (16). Therefore, during the season, an atopic patient is often in a continuous late phase characterized by nasal eosinophilia and complaints mainly of nasal obstruction

(8). Then, intermittent exposure to grass pollen results in attacks of sneezing, rhinorrhea, and increased nasal blockage, and sometimes even general malaise (hay fever). It is especially during these attacks that patients want fast relief and need fast-acting medication. This acute attack of nasal symptoms is more like the EPR. Therefore, understanding the pathogenic mechanism of the EPR will also provide important insight on a rational use of antiallergic therapy, as well as a way to assess objectively the efficacy of different antiallergic drugs.

We have previously reported that a significant increase in the concentrations of histamine (median: 214 ng/g), tryptase (28 μU/g), and LTC₄ (20 ng/g) in nasal secretions occurred 5 min after NAC, and this was accompanied by nasal symptoms such as sneezing (median: eight times) and unilateral and/or bilateral nasal obstruction (94% of the patients) (10). Meanwhile, the percentage of eosinophils and the eosinophil cationic protein concentration did not increase. These data strongly support the hypothesis of an important role for mast-cell activation during EPR. However, the correlation between the evolution of different nasal symptoms and the concentration of different mediators after NAC has not yet been studied in detail.

We found itching and sneezing to be the predominant nasal symptoms within 5 min after NAC. They could appear as early as of 20–30 s and had mostly disappeared approximately 5 min after NAC. The other symptoms of rhinorrhea (demonstrated by the increase of nasal secretions) and nasal obstruction (confirmed by an increase of nasal resistance) could also appear a few minutes after NAC. By median weight of secretion per minute, rhinorrhea reached its peak at 1–5 min after NAC, while the maximum value of nasal obstruction was reached about 10–30 min after NAC. In most of the patients, both symptoms could last for at least 1 h after NAC. Our previous study demonstrated that significant increases of unilateral nasal resistance (the more reacting side) could persist for about 4 h after the initial allergen challenge (17). These data demonstrated that the presence and severity of different nasal symptoms differ in time and duration after allergen exposure. Observation of symptoms of nasal obstruction and rhinorrhea shows that the EPR often lasts longer than 1 h.

Histamine and tryptase are known to be preformed mediators of mast cells, and they are generated after cell activation. Our data showed that a maximal increase in concentration of histamine was measured already 1 min after allergen challenge. The response of tryptase release from the mast cell seemed to occur slightly later than histamine. This finding illustrates again that the release mechanism of these mediators from the mast cells differs in time. It has been found that topical application of budesonide had a strong effect on tryptase and LTC₄ release, but not on histamine during the EPR (18). The release of histamine from the mast cell has been thought to be the result of direct activation of this cell by the antigen (19). In another experiment, we demonstrated that histamine release seems to be a very sensitive although quite nonspecific marker, as its concentration can also rise after nasal challenge with PBS in both normal test subjects and atopic patients. Tryptase proved to be a more specific marker of mast-cell activation in atopic patients during the EPR (20). LTC₄ is not stored in mast cells but is newly formed upon activation of the cells (21). Therefore, maximum increase of LTC₄ concentration in nasal secretions took slightly longer than histamine and tryptase after NAC. However, during the EPR, the mast cell is not the only cell type involved, for other cells can also participate in the production of inflammatory mediators.

We could not find significant correlation between the concentrations and dynamics of the mediators in nasal secretions, and the various nasal symptoms in these patients during the EPR. However, the time at which histamine concentrations

increase in the nasal secretions is generally associated with attacks of sneezing, and this happens within 1 min after NAC. The role of histamine in nasal allergic reaction has been confirmed by the reproduction of nasal symptoms after nasal provocation with histamine. These symptoms can be inhibited by the application of histamine antagonists. Histamine can stimulate trigeminal sensory nerves to produce itching nose and sneezing via central reflexes (22). LTC₄ is also an important inflammatory mediator which is thought to cause nasal obstruction and increased mucous production (21). Tryptase is an enzyme that reflects mast-cell degranulation. In addition, peptides such as calcitonin gene-related peptide (CGRP), substance P (SP), and neurokinin A (NKA) may participate in axon-response-mediated vasodilation and plasma extravasation during allergic reactions (23).

In summary, this study has demonstrated that the respective releases of mediators such as histamine, tryptase, and LTC₄ from mast cells differ slightly in time with respect to their peak concentrations in nasal secretions after NAC. A maximal increase of histamine concentration occurs within 1 min after NAC and is associated with attacks of itching and sneezing. If one looks only at the symptoms of rhinorrhea and nasal obstruction, the EPR often persists longer than 1 h.

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